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The Purity of Radioiodide-I^{131} Assessed by *in Vivo* and *in Vitro* Methods

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ABSTRACT

Between 41 and 94% of the radioactivity of 24 preparations of I^{131} supplied without cysteine preservative was non-iodide on chromatographic analysis. Extraneous radioactivity was essentially absent from I^{131} supplied with cysteine. It was converted to iodide-I^{131} by 10^{-3} M cysteine or iodide but not by incubation at pH 2. The average thyroid uptake of I^{131} containing extraneous radioactivity was significantly lower than the uptake of I^{131} free from non-iodide impurity in 16 human subjects measured under controlled conditions and in a random group of 669 patients. Incubation of samples of I^{131} containing non-iodide radioactivity with tyrosine and cupric chloride resulted in the non-enzymatic formation of monoiodotyrosine-I^{131} either in the presence or absence of thyroid homogenate. Enzymatic formation of monoiodotyrosine-I^{131} by thyroid homogenates could be demonstrated only when I^{131} free from extraneous activity was used.

RADIOIODIDE-I^{131} has proved to be one of the most valuable and extensively used of the radioactive isotopes. It has found widespread application in investigations of thyroid function and of thyroid hormone metabolism. It is of prime importance to investigators using I^{131} that the material be radioactively and chemically pure. That is, the carrier-free aqueous solution in which the isotope is supplied should contain iodine only in the form of iodide-I^{131}.

Recent publications[1-5] have reported that preparations of radioiodide-I^{131} supplied commercially without cysteine preservative may contain appreciable non-iodide radioactivity. The extraneous material in such preparations was believed to be radioactive iodine in some unidentified chemical form other than iodide. Treatment with acid,[3-5] cysteine,[1, 4, 5] or iodide[1] was said to convert the major contaminant to iodide-I^{131}. Thyroid uptake of the non-iodide radioactivity was reported lower than that of pure iodide-I^{131} in day-old chicks,[2] in rats,[5] and in surviving thyroid slices,[5] but not in three euthyroid human subjects.[3]

A project under way in our laboratory concerns the investigation of certain aspects of thyroid hormone metabolism using an *in vitro* technique involving incubation of homogenates of thyroid tissue with I^{131}. A failure to reproduce previously established results[6, 7] with this system led us, in the light of the aforementioned reports, to doubt the purity of the I^{131} being used. Analysis of the material by ascending filter paper chromatography revealed very appreciable amounts of non-iodide radioactivity in all samples tested. The results prompted us to investigate the chromatographic purity of all samples of I^{131} received by the University of Alberta Hospital between February and September 1961. In addition it was considered important to determine more fully the effect of non-iodide radioactivity on the *in vitro* studies which were in progress, and also to establish whether *in vivo* thyroid uptake measurements in human subjects were affected by the impure I^{131}. The purpose of this communication is to report the results of this study and to discuss certain differences in the chromatographic properties of the major non-iodide impurities in the preparations analyzed in our laboratory as compared to those reported by other workers.

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METHODS

Carrier-free solutions of I^{131} were obtained from two commercial sources. For purposes of conciseness these two preparations are identified as Product A and Product B throughout the rest of the paper. Product A was supplied by Charles E. Frosst & Co., Montreal, Quebec; Product B by Abbott Laboratories Ltd., Oak Ridge, U.S.A. According to the label, the latter contained 0.2% cysteine hydrochloride as preservative. The preparations were usually diluted with water to a suitable level of activity before use.

Paper chromatographic analyses were performed using an ascending technique in n-butanol-ethanol-2N ammonium hydroxide (5:1:2) or n-butanol-acetic acid-water (68:2:27) systems. The Whatman No. 1 filter paper strips were buffered to pH 7.4 with 0.067 M phosphate buffer. The chromatograms were radioautographed with Kodak "No-Screen" x-ray film to locate the position of radioactive spots.

A quantitative estimation of the radioactivity present in each spot on the chromatograms was obtained by cutting out the radioactive regions, using the corresponding autograph as a guide and counting each spot in a scintillation counter. The radioactivity in each position was expressed as a percentage of the total activity on the chromatogram.

Homogenates of rat thyroid tissue were prepared at a concentration of 5% in 1.15% potassium chloride from freshly excised or frozen glands in a Potter-Elvehjem homogenizer at 0° C. The supernatant remaining after centrifugation at 700xG for 10 minutes was used in the *in vitro* studies. Based on the technique described by Fawcett and Kirkwood,[6] 1.0 ml. aliquots of the thyroid homogenates were incubated with 1.0 ml. phosphate buffer, pH 7.4, 0.3 ml. 10^{-2}M copper chloride, 0.3 ml. 10^{-2}M tyrosine, 0.3 ml. 10^{-5}M potassium iodide, and 0.1 ml. (50 μc.) I^{131} in 15 ml. centrifuge tubes for one hour at 37° C. Inactivation of a thyroid preparation was attained by heating the homogenate in a boiling water bath for 10 minutes followed by chilling to 0° C. In control systems 1.0 ml. of 1.15% potassium chloride replaced the homogenate. At the end of the incubation period 20 μl. aliquots of each system were spotted directly on filter paper strips for chromatographic analyses. The positions of iodide, monoiodotyrosine and diiodotyrosine were identified on the chromatograms by adding small amounts of the stable compounds to the origin of each strip. The developed strips were sprayed with ammoniacal silver nitrate or with ninhydrin in butanol.

Measurements of the three-hour and 24-hour thyroid uptakes of I^{131} in human subjects were carried out following the routine used by the Radioisotope Laboratory of the University of Alberta Hospital. The I^{131} was given orally using a dose of 50 μc. in 50 ml. water. When more than one study was performed on the same individual, the doses of I^{131} administered were gradually increased from an initial 25 μc. to 50 μc. Residual thyroid activity was always measured prior to a second or third I^{131} administration; the residual counts corrected for physical decay were subtracted from subsequent readings. The detector consisted of a 1" x 1½" sodium iodide (TI) scintillation crystal mounted on a DuMont 6292 photomultiplier tube. Multiple one-minute counts were taken. The results exhibited a standard deviation in no single uptake of greater than 0.9% of the administered radioactivity.

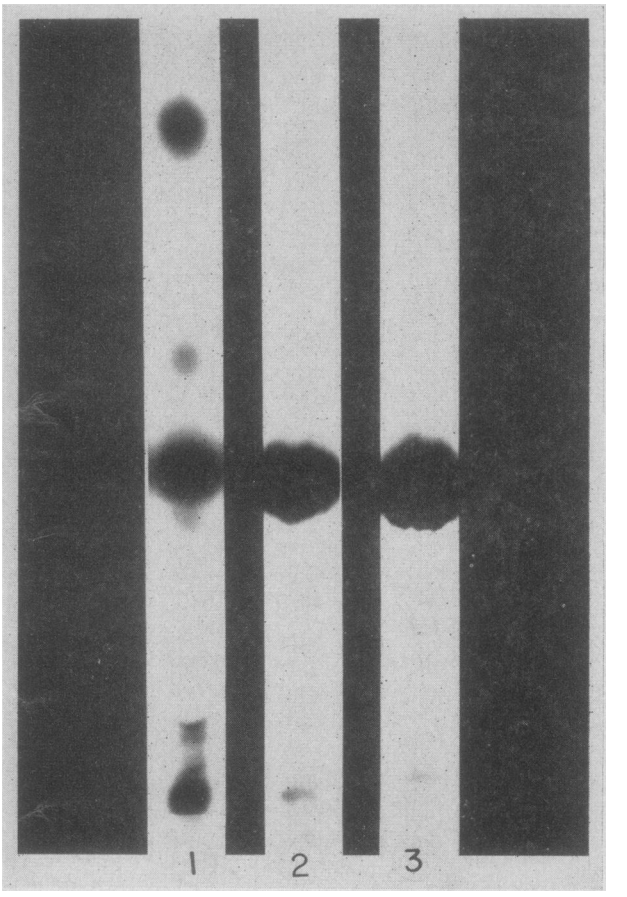

Fig. 1.—Radioautographs prepared from ascending paper chromatograms of I^{131} solutions: (1) Product A; (2) Product A after incubation with cysteine; (3) Product B. The solvent was butanol-ethanol-2N ammonia (5:1:2). The spot near the centre of each strip corresponds to iodide-I^{131}. All other spots correspond to extraneous non-iodide radioactivity.

RESULTS AND DISCUSSION

Fig. 1 illustrates three radioautographs of paper chromatograms each prepared from 50 μc. of an I^{131} preparation. The first autograph (1) is typical of the results obtained from commercial samples of radioactive iodine containing no cysteine preservative (Product A). The origin appears at the bottom, the solvent front at the top, and iodide near the centre of each strip. All spots other than that of iodide correspond to extraneous radioactivity. The second strip (2) illustrates that incubation of Product A with small amounts of cysteine (40 mg./ml.) for one hour at 37° C. causes most of the extraneous activity to revert to iodide-I^{131}. The final strip (3) illustrates analysis

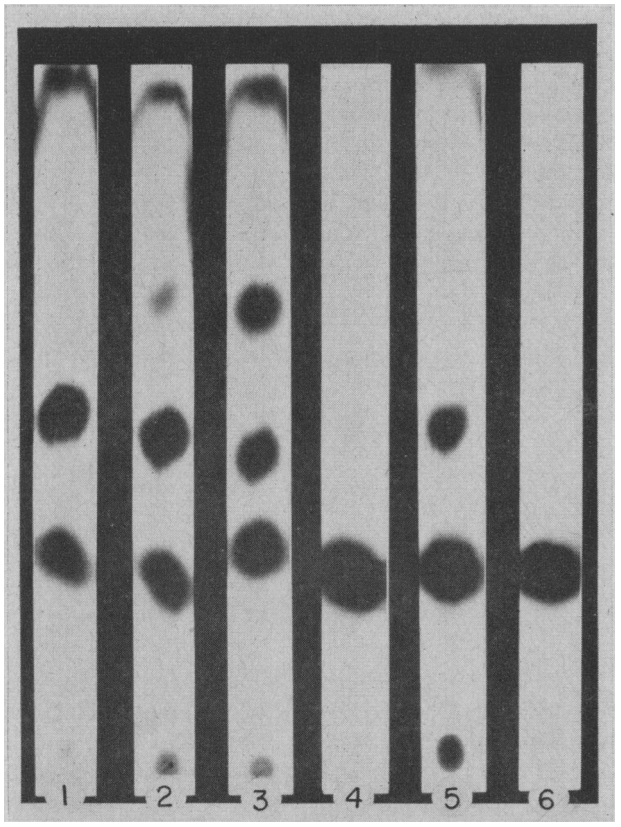

Fig. 2.—Radioautographs of ascending paper chromatograms developed in butanol-acetic acid-water (68:2:27). The effect of non-iodide radioactivity on the *in vitro* formation of monoiodotyrosine-I^{131} in rat thyroid homogenates incubated with tyrosine, copper and I^{131} is illustrated. The I^{131} used in systems (1) to (3) was Product A, while Product B was used in the identical systems (4) to (6), respectively. (1) and (4) represent controls containing no homogenate; (2) and (5) represent active homogenate, and (3) and (6) heat-inactivated homogenate. Iodide-I^{131} corresponds to the first spot above the origin in each strip and monoiodotyrosine-I^{131} to the second. Other spots correspond to unidentified I^{131}-containing materials.

of a second commercial I^{131} preparation (Product B) which is supplied with cysteine preservative. It showed relative absence of non-iodide radioactivity.

Table I summarizes the average results obtained by quantitative chromatographic analysis of 24 samples of Product A, five samples of cysteine-treated Product A, and five samples of Product B. Aliquots of 50 μc. were used in all cases. The mean values are listed in the table in the same order as the activity appeared on the chromatograms. The ranges of values, which were found to be extremely variable from one sample of Product A to another,

TABLE I.—ANALYSIS OF I^{131} BY ASCENDING PAPER CHROMATOGRAPHY IN BUTANOL:ETHANOL:2N AMMONIA (5:1:2)

Position on chromatogram	*Product A*	*Product A + cysteine*	*Product B (contains cysteine)*
	(24 samples)	*(5 samples)*	*(5 samples)*
1 (origin)	3 (0- 9)*	2 (0- 8)	1 (0- 2)
2	28 (5-94)	1 (0- 2)	2 (0- 4)
3	7 (0-34)	1 (0- 2)	1 (0- 2)
4 (iodide)	33 (6-59)	88 (74-97)	91 (83-99)
5	5 (0-19)	4 (0-22)	4 (1- 8)
6	3 (0-14)	—	—
7 (solvent front)	21 (0-69)	4 (0-12)	1 (0- 2)

*Average percentage of total radioactivity on the chromatograms. Range shown in parentheses.

are given in parentheses. Position 1 corresponds to the origin, position 4 to inorganic iodide, position 5 to the material designated as "U" by Taurog[5] or "S" by Ahn and Rosenberg[4] and position 7 to the solvent front. On the average only 33% of the total radioactivity of Product A behaved as inorganic iodide. No sample tested gave a value for iodide of greater than 60%. Positions 2 and 7 correspond to the major regions of non-iodide radioactivity. In only a few samples did position 5 contain appreciable radioactivity. Following cysteine treatment of Product A, an average of 88% of the activity now behaved as iodide. This is very similar to Product B, in which an average of 91% of the activity appeared in the iodide spot.

Homogenates of thyroid tissue when incubated with tyrosine, copper and I^{131} synthesize monoiodotyrosine-I^{131}. The chromatograms corresponding to the autographs illustrated in Fig. 2 were prepared from such systems. I^{131} containing a majority of its activity in non-iodide form (Product A) was used as tracer in the systems represented by the three autographs on the left. I^{131} containing less than 10% non-iodide radioactivity (Product B) was used for the series of three shown on the right. In all other respects the systems were identical. The first spot above the origin in each autograph corresponds to iodide-I^{131}, the next to monoiodotyrosine-I^{131}. Autographs 1 and 4 represent synthesis of monoiodotyrosine-I^{131} in a control system without homogenate. Autographs 2 and 5 represent systems containing active homogenates, and 3 and 6 represent systems containing heat-inactivated homogenate. The previously established results[6, 7] agree with those shown on the right, that is, an enzyme-catalyzed synthesis of monoiodotyrosine-I^{131}. When I^{131} containing extraneous radioactivity was used, as shown on the left, there was a large non-enzymatic synthesis of monoiodotyrosine-I^{131} and the appearance of unknown substances in the system.

To determine the effect of non-iodide radioactivity on the thyroid uptake of I^{131} in humans, comparative tests were performed using 16 euthyroid adult subjects. For each test 25 μc. of one I^{131} preparation was given orally and approximately one week later 50 μc. of a second preparation was administered to the same subjects. The thyroid uptake of the I^{131} was measured usually at three hours and in all cases at 24 hours.

Fig. 3 illustrates the 24-hour thyroid uptakes of Product A and of Product B in the same eight individuals. The clear bars represent Product A, the shaded bars Product B. Product B was found to result in a higher thyroid uptake than Product A in all subjects. The corresponding three-hour thyroid uptakes in seven of the same individuals are not illustrated, but in all but one case were significantly greater when Product B was used for the test.

In a similar manner the thyroid uptake of Product A was compared to that of Product A which had

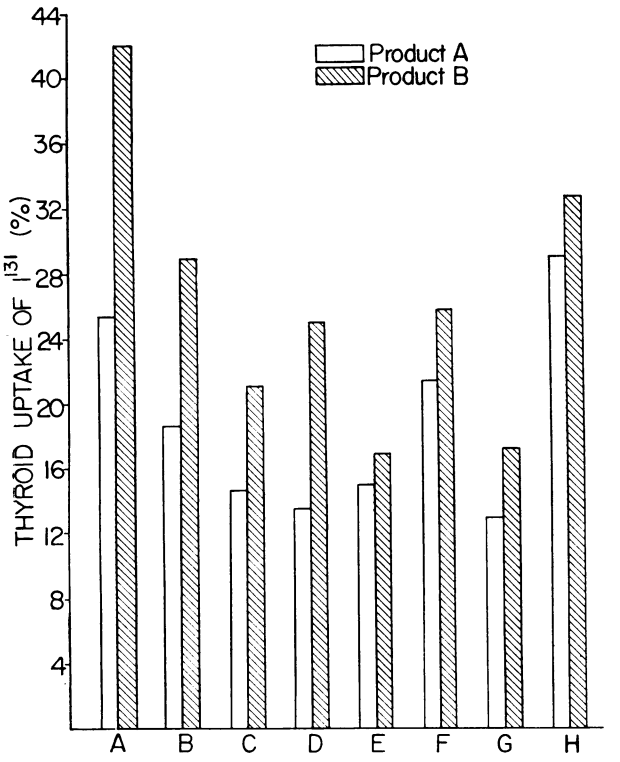

Fig. 3.—A comparison of the 24-hour thyroid uptake of Product A and of Product B in eight human subjects. Product B is supplied with cysteine preservative.

been treated with cysteine. A second group of eight subjects was used for this test. Fig. 4 compares the 24-hour thyroid uptakes of I^{131} measured in these individuals. In six of the eight subjects a significantly greater uptake of I^{131} followed cysteine treatment of the radioactive preparation. The three-hour thyroid uptakes which were measured in five

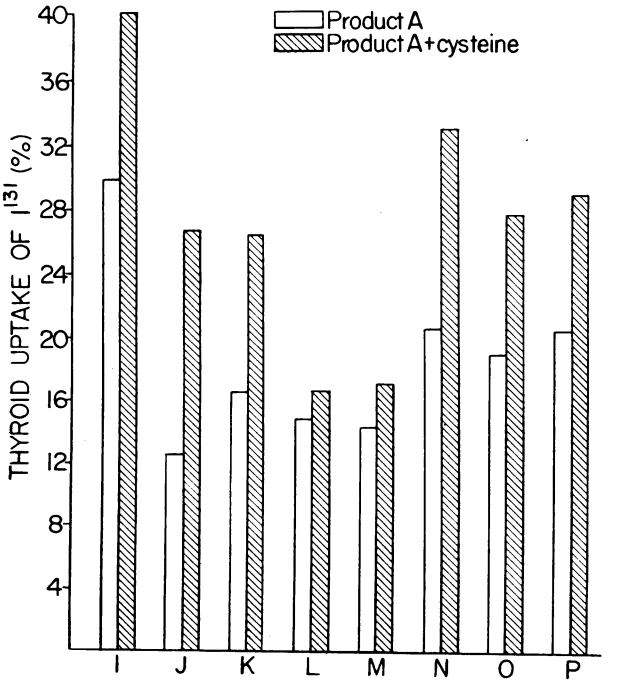

Fig. 4.—A comparison of the 24-hour thyroid uptake of Product A and of cysteine-treated Product A in eight human subjects.

of the same eight subjects were all higher when the treated material was given.

The average numerical values of the thyroid uptakes from the two series are summarized in Table II. To determine the statistical significance of the result, the "t-test" for differences was applied. The differences between the 24-hour thyroid uptakes were significant at the 1% level, and those of the three-hour uptakes were significant at the 5% level.

TABLE II.—THYROID UPTAKE OF I^{131} IN HUMAN SUBJECTS (AVERAGE VALUES)

	Uptake in 3 hrs.	*Uptake in 24 hrs.*
	(%)	(%)
Product A	7.1	18.9
Product B (contains cysteine)	12.0 (7 subjects)	26.2 (8 subjects)
Product A	6.1	18.5
Product A + cysteine	10.1 (5 subjects)	26.8 (8 subjects)

The routine I^{131} thyroid uptake tests, which were carried out at the University of Alberta Hospital during the period May to November 1961, made use of Product A as I^{131} tracer. The mean 24-hour thyroid uptake in 500 consecutive patients measured during this period was calculated and found to be 26.49%, with a standard deviation of 19.23%.

Beginning in December 1961, a change was made to Product B for routine thyroid uptake measurements in the same laboratory. To date 169 patients have been tested since the change occurred. The mean 24-hour thyroid uptake in this group was found to be 33.08%, with a standard deviation of 23.00%. The "t-test" was applied to these values and the difference was found to be significant ($P < 0.001\%$). The subjects of either series were not selected in any way and should represent a similar random distribution of thyroid function. The 500 values making up the first group were subdivided into five sets of 100 consecutive readings each. The mean and standard deviation of each set were determined. The values were 28.70 ± 20.85, 24.14 ± 17.41, 24.75 ± 18.27, 28.01 ± 20.03, and 26.85 ± 18.97, respectively. The "t-test" was applied to all possible paired combinations of these values. No significant difference occurred between any pair when analyzed in this manner.

The proportional increase in iodide-I^{131} which results from incubation of I^{131} preparations containing appreciable extraneous activity with increasing concentrations of cysteine hydrochloride is illustrated in Fig. 5. Presumably the action of cysteine on the extraneous material is that of a reducing agent.

Similarly the effect of iodide concentration on the extraneous radioactivity in I^{131} preparations was studied. As reported by previous investigators,[1] relatively high concentrations of iodide were found to cause most of the radioactive material to behave

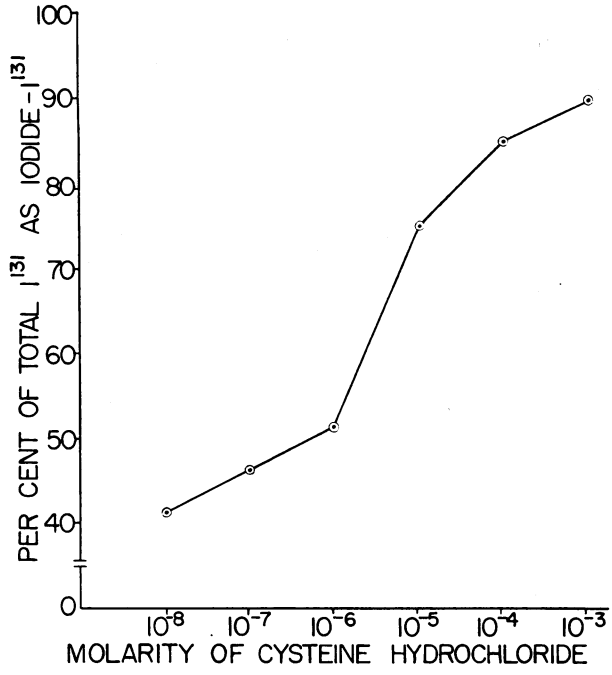

Fig. 5.—The effect of cysteine hydrochloride on non-iodide radioactivity in Product A-I^{131}.

as iodide-I^{131} on chromatography. Fig. 6 illustrates the gradual decrease in non-iodide radioactivity which occurred when aliquots of an I^{131} preparation were incubated at 37° C. for one hour with stable potassium iodide ranging in final concentration from 10^{-9}M to 10^{-2}M. At iodide concentrations above 10^{-4}M over 90% of the radioactivity behaved as iodide-I^{131} on chromatography.

The similarity between the action of cysteine and iodide suggests that the iodide, like the cysteine, chemically reduces the extraneous materials to iodide-I^{131}. Very little change in the chromatographic pattern of the I^{131} occurred when small amounts of stable iodide were added to the preparations, making it unlikely that the change which iodide brings about is due to an exchange type of reaction.

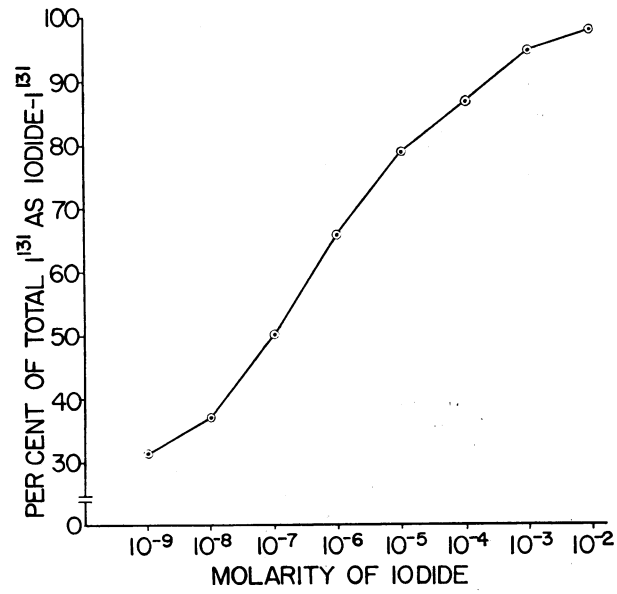

Fig. 6.—The effect of iodide on non-iodide radioactivity in Product A-I^{131}.

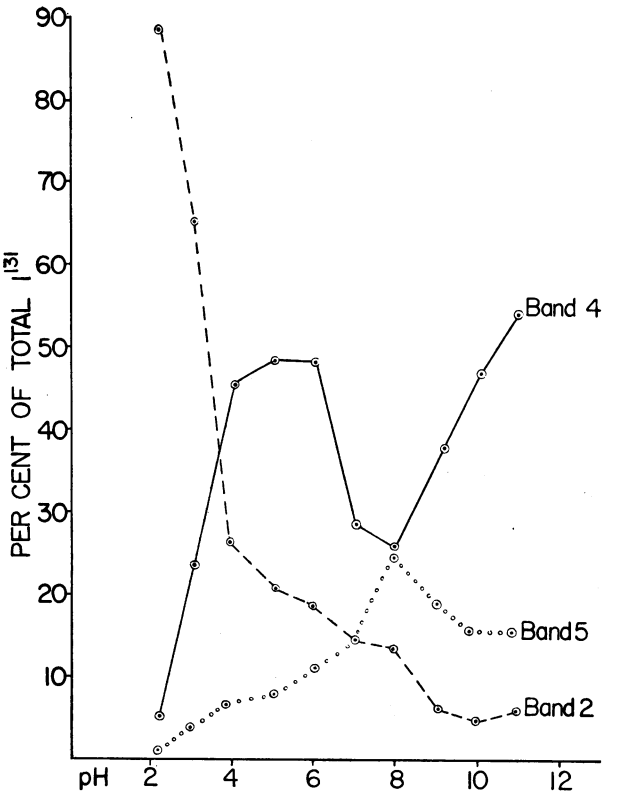

Fig. 7.—The effect of pH on extraneous radioactivity in Product A-I^{131} as analyzed by paper chromatography in butanol-ethanol-2N ammonia (5:1:2). Band 2 corresponds to non-iodide radioactivity running slightly above the origin of the chromatograms. Band 4 corresponds to iodide-I^{131}. Band 5 corresponds to extraneous radioactivity with similar properties to compound "U" described by Taurog.[5]

Previous workers[3, 5] have reported that exposure of "impure" I^{131} preparations to an acid pH (2-3) converts all but a small proportion of the extraneous material ("U") to iodide-I^{131}. Fig. 7 illustrates the changes which occurred when a sample of Product A was incubated at 37° C. for one hour at pH's varying from 2 to 11. The solutions were neutralized before chromatography. A sample of I^{131} was chosen which initially contained a relatively high proportion of Taurog's "U", that is material running at position 5 in our solvent. As was reported by Taurog,[5] a low pH almost completely eliminated "U". However, with Product A, as the pH was lowered, there was found to occur a very marked increase in the extraneous material running at position 2. At pH 2.0 nearly 90% of the total radioactivity appeared in Band 2. It has been postulated[3] that administration of I^{131} by the oral route would likely expose the extraneous radioactivity to a low pH in the stomach, with a presumed change to iodide-I^{131} occurring. If the only or major extraneous material occurring in the preparation were "U", this would be a reasonable supposition. However, the preparations that have been studied in this investigation have contained, on the average, only 5% "U" and an average of 28% material running at position 2. In this type of

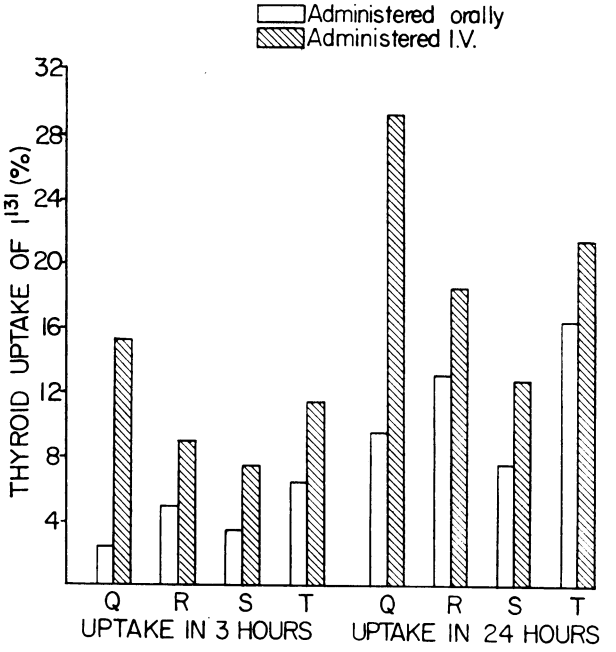

Fig. 8.—The three-hour and 24-hour thyroid uptakes of Product A in four human subjects, comparing the results after oral and intravenous administration of the I^{131}.

preparation the low pH of the gastric contents would be predicted to actually increase the proportion of non-iodide radioactivity.

Four euthyroid individuals were given intravenously 25 μc. of Product A, and the three-hour and 24-hour thyroid uptakes of I^{131} were measured. One week later the same subjects were given 50 μc. of the same preparation of Product A by oral administration. The thyroid uptakes were again measured, suitable corrections for residual activity and decay being made. The results are plotted in Fig. 8. In all cases the uptakes measured after intravenous administration of the I^{131} were higher than those after oral ingestion of the tracer. Such a difference was not observed in a parallel experiment using Product B in two subjects.

None of the extraneous bands was found to be identical chromatographically to stable iodate or periodate in the solvents used in this study. Band 2 material runs very close to added iodate, but can be distinguished from it. The very appreciable non-enzymatic formation of monoiodotyrosine-I^{131} which occurs when tyrosine is incubated with Product A suggests that at least a part of the extraneous radioactivity is in a reactive oxidized form of iodine similar to iodate.

SUMMARY

Unusual results which were obtained during *in vitro* studies of thyroid iodine metabolism have led to an investigation of the purity of commercial samples of I^{131}.

An analysis of over 30 I^{131} preparations by paper chromatography has shown that samples supplied without cysteine preservative contain appreciable quantities of non-iodide radioactivity. Commercial samples containing cysteine preservative were found to be relatively free of impurity.

Incubation of thyroid homogenates and tyrosine with impure samples of I^{131} led to the formation of monoiodotyrosine-I^{131}, but the iodination which occurred was found to be largely non-enzymatic. The expected enzymatic formation of monoiodotyrosine-I^{131} in such systems occurred only when cysteine-treated I^{131} was used.

Consistently lower thyroid uptakes of I^{131} were found in human subjects following administration of I^{131} containing extraneous non-iodide activity than when cysteine-treated I^{131} was given. This difference was observed both in a relatively small group where the same 16 subjects were used for both measurements under controlled conditions and in a large random group where 669 consecutive routine thyroid uptake readings were analyzed.

The previously reported conversions of non-iodide radioactivity in commercial samples of I^{131} to iodide-I^{131} by stable iodide and by cysteine were confirmed. However, unlike other investigations in which a low pH was reported to eliminate extraneous activity, incubation at acid pH of the I^{131} preparations studied in this investigation was associated with a marked increase in non-iodide radioactivity.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

There is an old saying that a man should always tell the truth to his banker and to his physician. A man who lies to his banker is apt to find himself in gaol. The penalty for lying to a physician is not prescribed in the code. A certain amount of confidence in the word of the individual is the basis of all society and the foundation of every day life. But it is not always possible to obtain tangible proof that a statement made is correct. Therefore, unless an individual is notoriously untruthful, or there is other grave reason for doubt, his given word is accepted. This fact is not recognized by the Toronto Street Railway Company, which, by a series of frauds, would seek to shake the very foundations of all social organizations. They would turn the machinery of justice into a jest; they would deceive the medical examiner, and so make it impossible for him to make a correct and just diagnosis; they would make the individual into a cheat, and all this to serve their own ends and make it impossible for a person who has been injured in any way by or through that company to demand just compensation.—Editorial, *Canad. Med. Ass. J.*, 2: 423, 1912.